Research Paper

Formulation of Co-Crystal in the treatment of Insulin Resistance NAFLD

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ABSTRACT

A principal pathological factor affecting NAFLD's (nonalcoholic fatty liver disease) onset and progression is insulin resistance; therefore, therapeutic strategies need to be developed that possess greater efficacy and bioavailability than current therapies. In this study, we examined the formulation and evaluation of pharmaceutical co-crystals of an antidiabetic agent (silymarin) with an appropriate co-former to modify their physicochemical and biopharmaceutical properties.

Co-crystals were prepared using an appropriate method such as solvent evaporation. Co-crystal formation was confirmed by the use of fourier transform infrared (FTIR) spectroscopy, which showed that the drug and co-former were interacting via intermolecular forces, including hydrogen bonding, as evidenced by the characteristic shift of corresponding peaks in both the drug and co-former spectra. Additionally, melting point analysis demonstrated unique thermal characteristics of the co-crystal compared to those of the pure components.

The results of an in vitro dissolution study showed that the co-crystal formulation enhanced the rate of dissolution and the amount of drug released compared to the pure drug and the physical mixture. This enhancement was likely due to the presence of different crystal lattice structures, a reduction in the amount of crystalline material present, and an improvement in the wettability of the drug.

Keywords: Co-crystal, FTIR, Hydrogen bonding, In-Silico study, Solvent Evaporation, In Vitro Dissolution.

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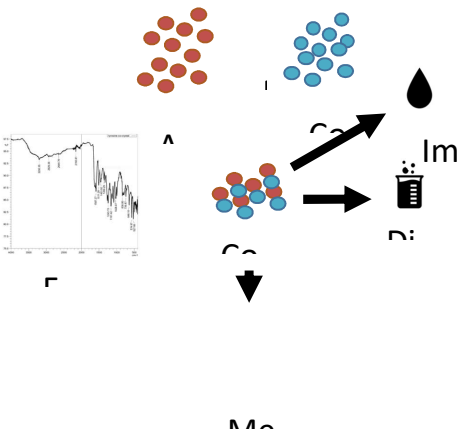


1. INTRODUCTION

Pharmaceutical researchers are continually in work on modifying the physicochemical properties of the drug substances to enhance the therapeutic results of the drug. The enhancement in physico-chemical properties, such as solubility, stability, etc., will be obtained by making numerous dosage forms of the drug.**QianoN.et al**. [1]

The FDA defines co-crystals as "a substance made up of two or more different molecules in a uniform ratio held together in a single crystalline lattice by non-ionic and non-covalent forces." Co-crystal components can be highly soluble and normally come from organic GRAS materials. **Bidhuri NA.et al**. [2] Many commonly used medicines are associated with challenges surrounding their direct delivery by mouth because they don't dissolve well in water, or they cannot pass through membranes easily. Co-crystal formation is a cost-effective and efficient way to enhance those characteristics of drug products, thus resulting in higher solubility, stability, and oral bioavailability of steam. **Sarathi P.et al**. [3][4] Co-crystallization is a method of producing multicomponent co-crystal system in which API and co-crystal former or held together via non-covalent interactions, H-bond or pi-pi interactions. **Verma S.et**

al.[5][6] Co-crystal formers are safe and pharmacologically inactive compounds that generally improve the physicochemical properties of the drug. **Chhajed, Santosh Subhash, et al**. [7] Synthesis of co-crystals depends on the functional group of API and co-crystal formers to allow for the occurrence of H-bond, pi-pi interaction or other forms of solid interactions. **Sangtani E.et.al** [8] Molecular docking is an emerging technique for pharmaceutical co crystal study and selection of suitable co-former for co-crystallisation of an API. **Budiman A.et al**. [9] Cocrystal Engineering has available an array of application ranges for physicochemical property improvement over co-crystal formation. Enhancements in the solubility, stability, bioavailability and mechanical properties have been recorded. **Miroshnyk I.et al**.[10] The purpose of this study is to create and evaluate an innovative method for creating pharmaceutical co-crystal formulations with antidiabetic drugs and co-formers that enhance the physicochemical properties, bioavailability, and therapeutic efficacy of these drugs in treating non-alcoholic fatty liver disease and its relationship to insulin resistance. The researcher will examine the synergistic effects between co-formers and drugs in reducing oxidative stress associated with both insulin resistance and non-alcoholic fatty liver disease (NAFLD).

MATERIALS USED

Silymarin was purchased from Sigma-Aldrich (Product of India). L-Tyrosine was purchased from Central Drug House (New Delhi). Methanol HPLC Grade was purchased from CDH(New Delhi). Hydrochloric acid was purchased from CDH(New Delhi).

In-Silico Screening

By using this computational process, a good coformer for an API could be located for efficient co-crystallisation, potentially through methods of binding affinity and hydrogen bonding. In order for co-crystalisation to take place efficiently, the in silico screening method follows a computerised process that selects those molecules from a library of available coformers with the highest binding affinity to the API. Virtual screening has also recently gained a lot more attention as a very useful way to successfully locate bioactive compounds. There are many in-silico screening platforms currently available, such as the Schrodinger Suite, PyRx, Open Babel Tool, Autodock 4.2.1, and BIOVIA Discovery Studio, among others, that have been specifically designed for the selection of the best coformer.

PREPARATION OF Co-CRYSTAL BY THE SOLVENT EVAPORATION METHOD

Co-crystal of Silymarin and L-Tyrosine was prepared in a ratio of 1:1 by the solvent evaporation technique. Weighed amounts of drug and coformer were mixed separately in the required amount of methanol, 30 ml . Coformer is then added slowly to the drug with mild agitation and heating at a magnetic stirrer for 1 hour at 100RPM. This ensures that the drug and conformer were properly mixed and no residue is left. The solvent is then left for 24 hours covered with aluminium foil for evaporation. After 24 hours, co-crystals were formed after evaporation of the organic solvent.

FTIR analysis

FTIR spectra were obtained for the drug and the prepared co-crystal. Spectra were recorded in the range of 4000-400 cm-1 by a PerkinElmer diamond ATR FTIR spectrophotometer (Shimadzu). Clean the ATR crystal and ATR crystal plate with an appropriate cleaner on a Kimwipe (e.g., isopropyl alcohol).

Melting point

The melting point of the drug and co-crystal was determined using LAB INDIA VISUAL MELTING RANGE. The sample was placed in a melting point capillary. These capillaries were placed vertically into the instrument the temperature was set to manual.

In-vitro Dissolution Study

Silymarin and produced co-crystals were studied in vitro in a USP type II dissolution apparatus (paddle type). As a dissolving medium, about 900ml of HCL buffer with a pH value of 2.1 was utilized independently. The medium temperature was set at 37.5°C, and the rotational speed of the paddle was set to 100rpm. To the dissolving media was added a sample of each preparation corresponding to a 50mg dosage of medication. At different predefined time intervals (30, 60, 90, 120, 150 min), 5ml of sample was taken from the dissolving medium, and 5ml of blank dissolution media was added after each withdrawal to replace the dissolution medium. All samples were filtered through a 0.45 µm filter before being analyzed using a UV spectrophotometer at 280nm. Comparative dissolutions of drugs from the co-crystals made with coformers were made against an untreated drug and physical mixtures of the drug with coformers. Each mixture's dissolution efficiency was calculated using Equation 1.

$$D = \frac{\int_{t1}^{t2} ydt \times 100}{y10\ (t2-t1)} \quad \text{eq1}$$

Similarity factor (f2)

This is a Factor of Similarity determined to illustrate if two comparative formulations are, indeed, similar. The FDA generally accepts a Factor of Similarity between 50-100% to be a 'similar calculation' according to this guideline. Similarity Factor can be calculated using Equation 2.

$$f2 = 50 \times log\left\{[1 + \left(\frac{1}{n}\right)\sum_{t=1}^{n} (Rt - Tt)^2]0.5 \times 100\right\} \quad \text{eq2}$$

The similarity factor is made up of 2 components: the number of dissolution samples collected over time (n) and the individual (or mean) percent dissolved at each time (Rt & Tt) of reference and test dissolution profiles. A similarity factor can only range from 0 to 100, with 100 meaning 2 reference/test groups are identical and 0 as they are increasingly dissimilar.

Difference Factor (f1)

The difference factor focuses on the difference in percent dissolved between the reference and test at various time intervals. It can be mathematically computed by using Equation 3

$f1 = \{[\sum_{t=1} \quad n|Rt - Tt|]/[\sum_{t=1} \quad nRt]. 100$ eq3

The f1 and f2 factors are used to compare differences between two drug dissolution profiles. The FDA has included f1 and f2 factors in numerous guidance documents, and they have offered specific criteria by which dissolution profiles may be evaluated for comparison.

1. Dissolution profiles must compare on the same number of units used (minimum 12), and similarity factors are only calculated when there is an average mean dissolution data with a sample size of 12 or more units for comparison. Mean data can only be used for the purpose of evaluating dissolution if the coefficient of variation is ≤ 20% at the first time point and ≤ 10% at all subsequent time points.
2. For accurate calculation of the similarity factor, a statistical approach of establishing confidence intervals to determine whether the reference and test are statistically significant or not may be used.
3. The dissolution conditions should be identical for both reference and test products (for example, the strength of the dosage form, test time intervals, temperature, rpm, total test time, etc.).
4. The literature also states to consider only one time after 85% dissolution of the product, since f2 values are sensitive to the number of dissolution time points
5. For rapid dissolving products that may dissolve 85% in 15 minutes, comparison of dissolution profiles is not mandatory**. Pravin Maurya et al. Yuksel N. et al**.[11],[12]

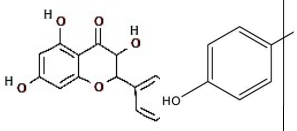
Result and Discussion

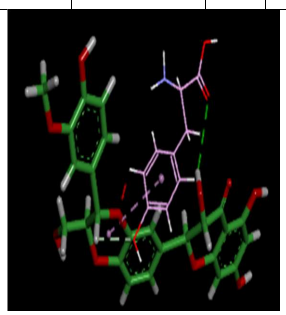
This chapter reports and discusses the findings of studies conducted on the hepatoprotective drug silymarin . This chapter contains the results of pre-formulation studies of the drug, as well as the characterization and evaluation of cocrystals. Where applicable, the data have also been statistically analyzed

In silico docking studies

Silymarin is mainly made up of its active component, silibinin. It is a polyfunctional flavonolignan with multiple hydroxyl (–OH), carbonyl (C=O), and ether (C–O–C) groups and has a rigid aromatic structure. Its chemical structure allows for silymarin to form possible hydrogen bonds with itself and with any other supramolecular item that it comes in contact with**.** Binding affinity parameters and types of interaction, such as H-bond, π-stacking, were used for the selection of coformers. L-Tyrosine is selected for its higher binding affinity.

Table 1: In silico screening of coformers with Silymarin

Coformers	**Chemical structure**	**Binding affinity Ei (kcal/mol)**	**Types of interaction**
Silymarin	 **L-Tyrosine**	-2.9	Hydrogen bonds and pi-stacking bonds



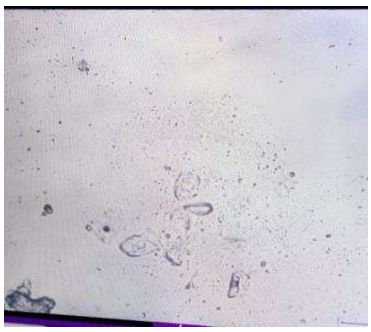


Fig 1 Binding site of silymarin with L-Tyrosine

Preparation of Co-Crystal

Co-crystal of silymarin and L-Tyrosine were prepared by the solvent evaporation method.

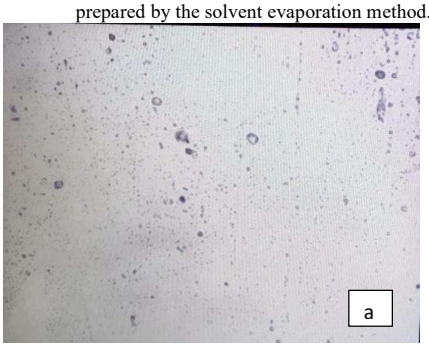


Fig 2(a): Co-crystal of Silymarin and L-Tyrosine and b: Silymarin drug

Characterization of Co-crystal

Prepared cocrystals were characterized by different analytical techniques such as FTIR and Dissolution.

FTIR:

The vibrational change in FTIR is important tool for confirmation of a cocrystal formation. The pure silymarin showed (–OH) stretching peak at 3451 cm^{-1} (C-H) stretching at 2936.15 cm^{-1} and (C=O) stretching at 1635 indicating strong intermolecular hydrogen bonding. In the cocrystal (–OH) stretching shifted at 3200 and 3393 cm^{-1} and ketone (C= O) stretching shifted at 1587 and 1511 cm^{-1} The shifting of –OH and carbonyl group (C= O) indicates that formation of a cocrystal.

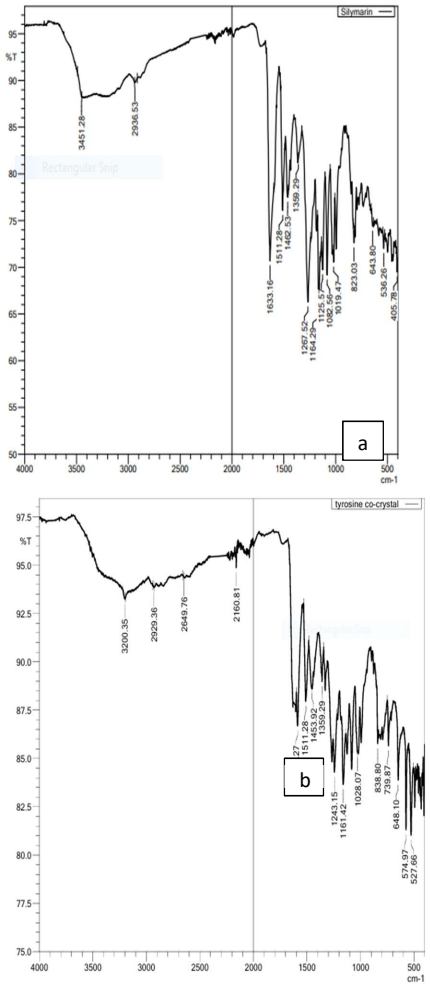


Fig 3(a) FTIR of Silymarin and (b) FTIR of Co-Crystal

In-vitro Dissolution study

The dissolution behavior of the co-crystal has greatly improved over that of the pure drug and demonstrates that there has been a successful modification of the physicochemical properties through co-crystallization. The improvement is thought to be mainly due to the change in the crystal lattice structure of the drug. The formation of a co-crystal creates new intermolecular interactions, including hydrogen bonds between the drug and the coformer, which lowers the lattice energy compared to the pure drug crystals. Hence, less energy

b

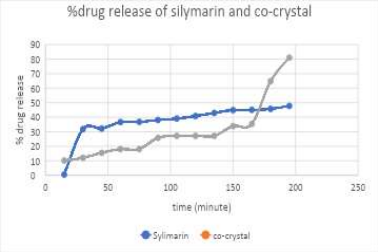
will be needed to disrupt the crystal lattice during dissolution, causing the dissolution rate to increase. Also, a hydrophilic coformer is present with the drug to enhance the wettability of the drug particle and increase its contact and interaction with the dissolution medium, which allows for quicker penetration of solvent molecules into the drug crystal surface resulting in faster drug release. Additionally, the formation of co-crystals can modify the crystal morphology and the surface characteristics of the particles, leading to an increase in the total effective surface area available for dissolution.

Overall, the improved dissolution behavior of the co-crystal can be explained by a combination of reduced lattice energy, enhanced wettability, increased surface area, and supersaturation effects, all of which collectively contribute to an increased dissolution rate as described by the Noyes–Whitney equation

$\frac{dQ}{dt} = \frac{DA(Cs-C)}{h}$ eq4

In order to compare the increase in dissolution ability of the co-crystal versus pure silymarin, both of their dissolution profiles were evaluated. The only significant point of concern was that the pure silymarin started dissolving very slowly and continued to do so until it reached 47.84% cumulate of drug released after 195 minutes, confirming the fact that it was poorly water-soluble and had low bioavailability due to the limitations of dissolution.

The co-crystal formulation had significantly increased dissolution characteristics compared to the other formulations, with an average of 81.0% drug release after 195 minutes. The co-crystal formulation displayed a marked increase in dissolution kinetics, particularly after minute 150mm. Disposal of the co-crystal enhanced crystalline drug-solvent interaction and improved the mass transfer. The above enhancements can be attributed to the disruption of the crystal lattice, the lowering of lattice energy, improved wettability, and possibly hydrogen bonding interactions between the drug and the co-former, which all contributed to a higher rate of drug dissolution. Quantitatively, the co-crystal formulation exhibited approximately 1.69-fold enhancement in cumulative drug release compared to the pure drug at the final time point.



The dissolution profiles of pure silymarin and its co-crystal formulation were compared using model-independent methods, namely the difference factor (f1) and similarity factor (f2). The calculated f1 and f2 values were found to be 43.48 and 38.14, respectively. According to regulatory criteria, f1 values between 0-15 and f2 values between 50-100 indicate similarity between dissolution profiles. However, the obtained values fall outside the acceptable limits, indicating a significant difference between the release profiles of the pure drug and co-crystal formulation. This deviation suggests that co-crystallization has substantially modified the dissolution behavior of silymarin, potentially due to improved wettability, reduced crystallinity, and enhanced intermolecular interactions, leading to altered drug release characteristics.

Conclusion

This research successfully showed how to create a co-crystal formulation of an antidiabetic medication to manage non-alcoholic fatty liver disease (NAFLD) that is related to insulin resistance. The data support the notion that using co-crystallization is an effective method for altering poorly soluble medications' physical and chemical features.

FTIR testing has been used to assess whether or not co-crystals were formed, and melting point tests have demonstrated that a new crystal was created, which is different from the parent compounds. The co-crystal formulation had an enhanced dissolution rate when compared to the pure drug or the physical mixture, indicating an increase in solubility.

Because of the greater dissolution profile observed in co-crystals compared to pure drugs, the co-crystal formulation is likely to be more effective than pure drug in producing therapeutic benefit, especially in the treatment of conditions such as insulin resistance, where drug absorption greatly affects therapeutic outcome. Furthermore, including the proper co-formers will probably yield synergistic benefits, for example, antioxidant effects that pertain to the pathophysiology of NAFLD.

In summary, co-crystal formulations are an innovative and useful method for improving drug performance.

Future in vivo studies are needed to verify the clinical applicability and therapeutic potential of these formulations for treating insulin resistance-related NAFLD.

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